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10/014,101	12/10/2001	Thomas Schmulling	1195-2	2633

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EXAMINER	
BAUM, STUART F	

ART UNIT	PAPER NUMBER
1638	

DATE MAILED: 01/12/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/014,101	SCHMULLING ET AL.	
	Examiner	Art Unit	
	Stuart F. Baum	1638	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 October 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *2-138* is/are pending in the application.

4a) Of the above claim(s) *See Continuation Sheet* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *2-4,7-17,25,28-44,46,47,49,50,52,53,79-81,86,87,90-92,95-101,103-121 and 138* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *10 December 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: *sequence search result*.

DETAILED ACTION

RCE Acknowledgment

1. The request filed on October 6, 2005 for a Request for Continued Examination (RCE) under 37 C.F.R. § 1.114, based on parent Application No. 10/014,101 is acceptable and a RCE has been established. An action on the RCE follows.

Claims 2-138 are pending.

Claim 1 has been canceled.

Claims 5-6, 18-24, 26-27, 45, 48, 51, 54-78, 82-85, 88-89, 93-94, 102 and 122-137 are withdrawn from consideration for being drawn to a non-elected invention.

2. Claims 2-4, 7-17, 25, 28-44, 46-47, 49-50, 52-53, 79-81, 86-87, 90-92, 95-101, 103-121 and 138 including SEQ ID NO:26 encoding SEQ ID NO:4 are examined in the present office action.

Claim objection

3. Claims 103-105 are objected to under 37 CFR 1.75 as being a substantial duplicate of claims 106-108, respectively. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k).

New Matter

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

4. Claims 2-4, 7-17, 25, 28-44, 46-47, 49-50, 52-53, 79-81, 86-87, 90-92, 95-101, 103-121 and 138 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims have been amended to recite "with the proviso that an isolated nucleic acid molecule encoding a cytokinin oxidase from corn (maize) is not included". Applicants fail to point to support for the phrase in the instant specification. Upon a cursory search of the specification, support could not be found. Applicants are required to point to support for "with the proviso that an isolated nucleic acid molecule encoding a cytokinin oxidase from corn (maize) is not included" or to amend the claims to delete the NEW MATTER.

Written Description

5. Claims 2-4, 7-17, 25, 28-44, 46-47, 49-50, 52-53, 79-81, 86-87, 90-92, 95-101, 103-121 and 138 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the

relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are drawn to methods comprising an isolated nucleic acid molecule comprising a DNA sequence as set forth in SEQ ID NO:26, or the complement thereof, an isolated nucleic acid molecule comprising the RNA sequence corresponding to SEQ ID NO:26 or the complement thereof, an isolated nucleic acid molecule specifically hybridizing to SEQ ID NO:26 or to the complement thereof, under medium stringency conditions as specified in claim 2c, a functional fragment of said nucleic acid molecule having the biological activity of a cytokinin oxidase, a nucleic acid encoding a protein with an amino acid sequence comprising the polypeptide as given in SEQ ID NO:32 and which is at least 70% similar to the amino acid sequence as given in SEQ ID NO:4, an isolated nucleic acid molecule encoding an immunologically active fragment or a functional fragment of a cytokinin oxidase encoded by a nucleic acid molecule as set forth in SEQ ID NO:26, or a immunologically active fragment or functional fragment of any of the above nucleic acid molecules, a nucleic acid molecule encoding a protein as defined in SEQ ID NO:4 or the complement thereof; or vector, host cell, composition or plant comprising said nucleic acid molecule.

The Office interprets an isolated nucleic acid molecule comprising 'a' DNA sequence as set forth in SEQ ID NO:26 to read on a large number of sequences because the Office interprets "an isolated nucleic acid molecule comprising 'a' DNA sequence set forth in SEQ ID NO:26" to encompass nucleic acids comprising any portion of SEQ ID NO:26, including any dinucleotide of SEQ ID NO:26.

The Office interprets "an isolated nucleic acid molecule comprising the RNA sequence '*corresponding*' to SEQ ID NO:26, or complement thereof" to encompass RNA molecules encoded by homologues of SEQ ID NO:26. Therefore claims 2b and 3b are drawn to a large number of sequences.

The Office interprets a nucleic acid molecule encoding '*a*' protein as defined in SEQ ID NO:4, or complement thereof, to read on a large number of sequences because the Office interprets "a nucleic acid molecule encoding '*a*' protein as defined in SEQ ID NO:4" to encompass nucleic acid encoding any portion of SEQ ID NO:4, including any two amino acids of SEQ ID NO:4.

Because Applicants have not defined "similar" and for purposes of Written Description, the Office interprets "similar" to encompass a multitude of sequences encoding any protein having cytokinin oxidase activity.

Applicants disclose "Six different genes were identified from Arabidopsis thaliana that bear sequence similarity to a cytokinin oxidase gene from maize" (page 86 of specification, Example 2). Applicants disclose that the Arabidopsis thaliana cytokinin oxidase-like protein 2 (AtCKX2) has a protein sequence of SEQ ID NO:4 which is encoded by the cDNA sequence listed as SEQ ID NO:26 (page 88 of specification, lines 3-13). Applicants present an alignment of four Arabidopsis CKX proteins and one CKX protein from maize, in which the conserved amino acids are indicated (Figure 2 of the Drawings).

The Office contends that the isolated cytokinin oxidase-like proteins isolated from Arabidopsis do not have the same activity/function because Applicants disclose that plant transformation with the different CKX cDNA's isolated from Arabidopsis, do not produce the

same result. Applicants disclose "The AtCKX1 transgenics have longer primary roots, more side roots and form more adventitious roots. AtCKX2 transgenics lack the enhanced growth of the primary root but form more side roots and lateral roots than WT" (page 107 of specification, Conclusion). Applicants also disclose "However, overexpression of the AtCKX3 gene in tobacco resulted in a stronger phenotype compared to AtCKX2" (page 109 of specification, #3). Applicants also disclose "The alterations were very similar, but not identical, for the different genes" and concluding by stating "Therefore, a particular cytokinin oxidase gene may be preferred for achieving the phenotypes that are described in the embodiments of this invention" (page 111 of specification, lines 10-20).

The Applicants do not identify essential regions of AtCKX2 protein encoded by SEQ ID NO:26, nor do Applicants describe any polynucleotide sequences that specifically hybridizes to SEQ ID NO:26 or to the complement thereof under medium stringency conditions as specified in claim 2c, or that encodes a functional fragment of said nucleic acid molecule having the biological activity of a cytokinin oxidase, or that encodes a protein with an amino acid sequence comprising the polypeptide as given in SEQ ID NO:32 and which is at least 70% similar to the amino acid sequence as given in SEQ ID NO:4, or that encodes an immunologically active fragment or a functional fragment of a cytokinin oxidase encoded by a nucleic acid molecule as set forth in SEQ ID NO:26, or a immunologically active fragment or functional fragment of any of the above nucleic acid molecules, or that encodes any protein as defined in SEQ ID NO:4.

The Federal Circuit has recently clarified the application of the written description requirement to inventions in the field of biotechnology. See University of California v. Eli Lilly and Co., 119 F.3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). In summary, the court

stated that a written description of an invention requires a precise definition, one that defines the structural features of the chemical genus that distinguishes it from other chemical structures. A definition by function does not suffice to define the genus because it is only an indication of what the gene does, rather than what it is. The court goes on to say, "A description of a genus of cDNAs may be achieved by means of a recitation of a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to members of the genus, which features constitute a substantial portion of the genus." *See University of California v. Eli Lilly and Co.*, 119 F.3d 1559; 43 USPQ2d 1398, 1406 (Fed. Cir. 1997).

Applicants fail to describe a representative number of polynucleotide sequences encoding an AtCKX2 protein falling within the scope of the claimed genus of polynucleotides which hybridize to SEQ ID NO:26, are divergent alleles, or encode any immunologically active fragment or any of the claimed embodiments as recited above. Applicants only describe a single cDNA sequence of SEQ ID NO:26 encoding the amino acid sequence of SEQ ID NO:4. Furthermore, Applicants fail to describe structural features common to members of the claimed genus of polynucleotides. Hence, Applicants fail to meet either prong of the two-prong test set forth by *Eli Lilly*. Furthermore, given the lack of description of the necessary elements essential for the AtCKX2 protein, it remains unclear what features identify an AtCKX2 protein encoded by SEQ ID NO:26. Since the genus of AtCKX2 proteins encoded by SEQ ID NO:26 has not been described by specific structural features, the specification fails to provide an adequate written description to support the breath of the claims.

Sequences that hybridize with SEQ ID NO:26 or which comprise any DNA sequence as set forth in SEQ ID NO:26 or the complement thereof or sequences which encode a polypeptide which is at least 70% similar to SEQ ID NO:4 encompass naturally occurring allelic variants, mutants of CKX2 protein encoded by SEQ ID NO:26, as well as sequences encoding proteins having no known CKX2 activity, of which Applicant is not in possession. Absent of such disclosure, one skilled in the art cannot determine the genus of sequences based upon the disclosure of the sequence of SEQ ID NO:26 with any certainty or predictability. Accordingly, the specification fails to provide an adequate written description to support Applicants' claim breadth. (See Written Description guidelines published in Federal Register/Vol. 66, No.4/Friday, January 5, 2001/Notices: p.1099-1111).

Applicant's arguments filed 7/7/2005 have been fully considered but they are not persuasive.

Applicants contend that "Figure 2 contains information about a dicot (Arabidopsis) and a monocot (maize)" (paragraph bridging pages 22 and 23 or Remarks). Applicants contend that "the sequence identity between AtCKX1 and AtCKX2 is as low as 38.2%, yet both proteins still function as cytokine oxidases and confer similar phenotype in the root" (*Ibid*).

The Office contends that the isolated cytokinin oxidase-like proteins isolated from Arabidopsis do not have the same activity/function because Applicants disclose that plant transformation with the different CKX cDNA's isolated from Arabidopsis, do not produce the same result. Applicants disclose "The AtCKX1 transgenics have longer primary roots, more side roots and form more adventitious roots. AtCKX2 transgenics lack the enhanced growth of the primary root but form more side roots and lateral roots than WT" (page 107 of specification,

Conclusion). Applicants also disclose "However, overexpression of the AtCKX3 gene in tobacco resulted in a stronger phenotype compared to AtCKX2" (page 109 of specification, #3). Applicants also disclose "The alterations were very similar, but not identical, for the different genes" and concluding by stating "Therefore, a particular cytokinin oxidase gene may be preferred for achieving the phenotypes that are described in the embodiments of this invention" (page 111 of specification, lines 10-20).

Scope of Enablement

6. Claims 2-4, 7-17, 25, 28-44, 46-47, 49-50, 52-53, 79-81, 86-87, 90-92, 95-101, 103-121 and 138 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods for stimulating root growth, enhancing lateral or adventitious root formation, increasing root size, the production of a transgenic plant and delaying the onset to flowering comprising transforming a plant with a nucleic acid molecule comprising SEQ ID NO:26 encoding the polypeptide comprising SEQ ID NO:4; vector and composition comprising said nucleic acid molecule, and isolated host cell, plant, plant cell or plant tissue transformed therewith, does not reasonably provide enablement for methods for effecting the expression of a polypeptide, increasing the size of the root meristem, altering leaf senescence, increasing leaf thickness, decreasing vessel size, improving standability, increasing branching, improving lodging resistance, increasing seed size or weight, increasing embryo size or weight, increasing cotyledon size, increasing yield, increasing growth of seedling or an increase in early vigor, or increasing stress tolerance comprising expression of any of the nucleic acid molecules of claim 3 or 4 or a nucleic acid molecule as defined in claim 2 and Applicants are not enabled for methods

for stimulating root growth, enhancing lateral or adventitious root formation, increasing root size, the production of a transgenic plant and delaying the onset to flowering comprising expression of a nucleic acid molecule of claim 3 or 4 or a nucleic acid molecule as defined in claim 2 with the exception of SEQ ID NO:26 encoding SEQ ID NO:4. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The claimed invention is not supported by an enabling disclosure taking into account the *Wands* factors. *In re Wands,* 858/F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988). *In re Wands* lists a number of factors for determining whether or not undue experimentation would be required by one skilled in the art to make and/or use the invention. These factors are: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples of the invention, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claim.

The claims are drawn to methods comprising an isolated nucleic acid molecule comprising a DNA sequence as set forth in SEQ ID NO:26, or the complement thereof, an isolated nucleic acid molecule comprising the RNA sequence corresponding to SEQ ID NO:26 or the complement thereof, an isolated nucleic acid molecule specifically hybridizing to SEQ ID NO:26 or to the complement thereof under medium stringency conditions as specified in claim 2c, a functional fragment of said nucleic acid molecule having the biological activity of a cytokinin oxidase, a nucleic acid encoding a protein with an amino acid sequence comprising the polypeptide as given in SEQ ID NO:32 and which is at least 70% similar to the amino acid

sequence as given in SEQ ID NO:4, an isolated nucleic acid molecule encoding an immunologically active fragment or a functional fragment of a cytokinin oxidase encoded by a nucleic acid molecule as set forth in SEQ ID NO:26, or a immunologically active fragment or functional fragment of any of the above nucleic acid molecules, a nucleic acid molecule encoding a protein as defined in SEQ ID NO:4; or vector, host cell, composition or plant comprising said nucleic acid molecule; and methods of stimulating root growth, enhancing lateral or adventitious root formation, stimulating root growth, increasing root size, the production of a transgenic plant and delaying the onset to flowering, effecting the expression of a polypeptide, increasing the size of the root meristem, altering leaf senescence, increasing leaf thickness, decreasing vessel size, improving standability, increasing branching, improving lodging resistance, increasing seed size or weight, increasing embryo size or weight, increasing cotyledon size, increasing yield, increasing growth of seedling or an increase in early vigor, or increasing stress tolerance comprising expression of said nucleic acid molecule.

The Office interprets an isolated nucleic acid molecule comprising 'a' DNA sequence as set forth in SEQ ID NO:26 to read on a large number of sequences because the Office interprets "an isolated nucleic acid molecule comprising 'a' DNA sequence set forth in SEQ ID NO:26" to encompass nucleic acids comprising any portion of SEQ ID NO:26, including any dinucleotide of SEQ ID NO:26.

The Office interprets "an isolated nucleic acid molecule comprising the RNA sequence '*corresponding*' to SEQ ID NO:26, or complement thereof" to encompass RNA molecules encoded by homologues of SEQ ID NO:26. Therefore claims 2b and 3b are drawn to a large number of sequences.

The Office interprets a nucleic acid molecule encoding '*a*' protein as defined in SEQ ID NO:4, or complement thereof, to read on a large number of sequences because the Office interprets "a nucleic acid molecule encoding '*a*' protein as defined in SEQ ID NO:4" to encompass nucleic acid encoding any portion of SEQ ID NO:4, including any two amino acids of SEQ ID NO:4.

Because Applicants have not defined "similar" and for purposes of enablement, the Office interprets "similar" to encompass a multitude of sequences encoding any protein having cytokinin oxidase activity.

Applicants disclose "Six different genes were identified from Arabidopsis thaliana that bear sequence similarity to a cytokinin oxidase gene from maize" (page 86 of specification, Example 2). Applicants disclose that the Arabidopsis thaliana cytokinin oxidase-like protein 2 (AtCKX2) has a protein sequence of SEQ ID NO:4 which is encoded by the cDNA sequence listed as SEQ ID NO:26 (page 88 of specification, lines 3-13). Applicants present an alignment of four Arabidopsis CKX proteins and one CKX protein from maize, in which the conserved amino acids are indicated (Figure 2 of the Drawings).

Applicants transformed Arabidopsis and tobacco with SEQ ID NO:26 operably linked to the CaMV 35S promoter (page 100, lines 9-27). Transformed plants exhibited a higher level of cytokinin oxidase activity (paragraph bridging pages 100-101). Applicants disclose that the transgenic plants were dwarfed (page 102, "Stem elongation" table) and plants exhibited more roots which are thick and formed aerial roots along the stem, a higher number of lateral and adventitious roots (page 102, "Roots"). The fresh and dry weight of root biomass of transgenic plants was greater than wild-type plants (page 103, lines 3 to page 104, line 15). Transgenic

plants also displayed a delayed onset to flowering and the number of flowers and the seed yield per capsule was reduced, but the weight of the individual seeds was not different when compared to seeds from wild type plants (page 104, lines 17-24). Applicants conclude by stating "The main difference between tobacco and Arabidopsis is the lack of enhanced primary root growth in AtCKX2 overexpressing plants" (page 107, lines 23-24). Applicants disclose that in transgenic tobacco, the primary root was longer compared to a non-transgenic control (page 102, lines19-20).

The Office contends that the isolated cytokinin oxidase-like proteins isolated from Arabidopsis do not have the same activity/function because Applicants disclose that plant transformation with the different CKX cDNA's isolated from Arabidopsis, do not produce the same result. Applicants disclose "The AtCKX1 transgenics have longer primary roots, more side roots and form more adventitious roots. AtCKX2 transgenics lack the enhanced growth of the primary root but form more side roots and lateral roots than WT" (page 107 of specification, Conclusion). Applicants also disclose "However, overexpression of the AtCKX3 gene in tobacco resulted in a stronger phenotype compared to AtCKX2" (page 109 of specification, #3). Applicants also disclose "The alterations were very similar, but not identical, for the different genes" and concluding by stating "Therefore, a particular cytokinin oxidase gene may be preferred for achieving the phenotypes that are described in the embodiments of this invention" (page 111 of specification, lines 10-20).

Re: Claims 56, 90-92, 95-97, 103-108, 114-115 and 119. Applicants fail to provide guidance for methods of increasing size of the root meristem, altering leaf senescence, increasing leaf thickness, decreasing vessel size, improving standability of seedlings, increasing branching,

improving lodging resistance, increase seed size or weight, increase embryo size or weight, increase cotyledon size, increase in growth of seedlings or an increase in early vigor, increase in yield, and increase in stress tolerance comprising transforming a plant with SEQ ID NO:26 encoding SEQ ID NO:4, or transforming a plant with any nucleic acid recited in any one of claims 2, 3 or 4. In fact, Applicants disclose that plants transformed with SEQ ID NO:26 had smaller leaves (page 106, bottom table) and seeds whose weight was not statistically different from untransformed seeds (page 105, bottom table). Applicants have not disclosed how decreasing cytokinin levels increases leaf thickness or how yield is increased even though plants produce less seeds, smaller leaves and flowers size is not changed.

Re: claims 28-29 recite a method for effecting the expression of a polypeptide comprising introducing a nucleic acid molecule of claim 3 or 4 into a plant. The recitation "effecting the expression" reads on methods not disclosed by applicants, e.g., altering expression of enhancers and/or suppressors. Applicants are not enabled for the full breadth of the claim.

The state-of-the-art teaches that not all cytokinin oxidases are the same. Kaminek et al (1990, Plant Physiol. 93:1530-1538) teach the isolated cytokinin oxidases from callus cultures of *Phaseolus vulgaris* L. cv Great Northern and *Phaseolus lunatus* L. cv Kingston have different enzyme activities and the pH optimum for cytokinin oxidase from *Phaseolus vulgaris* L. cv Great Northern is 6.5 whereas the pH optimum for cytokinin oxidase from *Phaseolus lunatus* L. cv Kingston is 8.4. Hare et al (1994, Physiologia Plantarum 91:128-136) teach that substrate specificity varies; cytokinin oxidase from the moss *Funaria* has a high affinity for the cytokinin kinetin, whereas most plant cytokinin oxidases do not have a high affinity for kinetin. They further report that cytokinin oxidases from *Dictyostelium discoideum* and *saccharomyces*

cerevisiae have a broader substrate specificity than most plant cytokinin oxidases (page 131, right column, 1st paragraph).

Applicants claims are drawn to nucleic acid sequences encoding functional fragments of a cytokinin oxidases, or immunologically active fragments of a cytokinin oxidase, or nucleic acids that are diverged from SEQ ID NO:26. The state-of-the-art is such that one of skill in the art cannot predict which nucleic acids that exhibit less than 100% sequence identity to SEQ ID NO:26 will encode a protein with the same activity as a protein encoded by SEQ ID NO:26. The prediction of protein structure from sequence data and, in turn, utilizing predicted structural determinations to ascertain functional aspects of the protein, is extremely complex, and the positions within the protein's sequence where amino acid substitutions can be made with a reasonable expectation of maintaining function are limited (Bowie et al, Science 247:1306-1310, 1990, see especially page 1306). Proteins may be sensitive to alterations in even a single amino acid in a sequence. For example, the replacement of a glycine residue located within the START domain of either the PHABULOSA or PHAVOLUTA protein receptor with either an alanine or aspartic acid residue, alters the sterol/lipid binding domain (McConnell et al, Nature 411 (6838):709-713, 2001, see especially page 710, left column, 2nd paragraph).

Applicants claims are drawn to nucleic acid sequences that hybridize to SEQ ID NO:26, but the state-of-the-art teaches isolating DNA fragments even using stringent hybridization conditions, does not always select for DNA fragments whose contiguous nucleotide sequence is the same or nearly the same as the probe. Fourgoux-Nicol et al (1999, Plant Molecular Biology 40 :857-872) teach the isolation of a 674bp fragment using a 497bp probe incorporating stringent hybridization conditions comprising three consecutive 30 minute rinses in 2X, 1X and 0.1X SSC

with 0.1% SDS at 65^{0}C (page 859, left column, 2nd paragraph). Fourgoux-Nicol et al also teach that the probe and isolated DNA fragment exhibited a number of sequence differences comprising a 99bp insertion and a single nucleotide gap, while the DNA fragment contained 2 single nucleotide gaps and together the fragments contained 27 nucleotide mismatches. Taking into account the insertions, gaps and mismatches, the longest stretch of contiguous nucleotides to which the probe could hybridize consisted of 93bp of DNA (page 862, Figure 2).

Applicants have not disclosed how one makes or isolates any of the sequences that are encompassed by Applicants' broad claims. Applicants have not taught which regions of the respective polynucleotides can be used to amplify any of said polynucleotides or which regions can be used as a probe to isolate any of said polynucleotide sequences.

In the absence of guidance, undue trial and error experimentation would be required for one of ordinary skill in the art to screen through the multitude of non-exemplified sequences, either by using non-disclosed fragments of SEQ ID NO:26 as probes or by designing primers to undisclosed regions of SEQ ID NO:4 and isolating or amplifying fragments, subcloning the fragments, producing expression vectors and transforming plants therewith, in order to identify those, if any, that when over-expressed have the activity of the protein encoded by SEQ ID NO:26 and produce the claimed phenotypes.

Therefore, given the breadth of the claims; the lack of guidance and examples; the unpredictability in the art; and the state-of-the-art as discussed above, undue experimentation would be required to practice the claimed invention, and therefore the invention is not enabled.

Applicant's arguments filed 7/7/2005 have been fully considered but they are not persuasive.

Applicants contend the different Arabidopsis AtCKX proteins all encode proteins having cytokine oxidase activity (page 24, top paragraph). Applicants contend the phenotypes observed for the different AtCKX transgenics are very similar (page 24, 1st full paragraph).

The Office contends that the different Arabidopsis AtCKX proteins may encode proteins having cytokinin oxidase activity, but Applicants own admitted statements disclose that the different AtCKX proteins do not produce the same phenotype when transformed into a plant. The Office contends that the isolated cytokinin oxidase-like proteins isolated from Arabidopsis do not have the same activity/function because Applicants disclose that plant transformation with the different CKX cDNA's isolated from Arabidopsis, do not produce the same result. Applicants disclose "The AtCKX1 transgenics have longer primary roots, more side roots and form more adventitious roots. AtCKX2 transgenics lack the enhanced growth of the primary root but form more side roots and lateral roots than WT" (page 107 of specification, Conclusion). Applicants also disclose "However, overexpression of the AtCKX3 gene in tobacco resulted in a stronger phenotype compared to AtCKX2" (page 109 of specification, #3). Applicants also disclose "The alterations were very similar, but not identical, for the different genes" and concluding by stating "Therefore, a particular cytokinin oxidase gene may be preferred for achieving the phenotypes that are described in the embodiments of this invention" (page 111 of specification, lines 10-20).

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

7. Claims 10-15, and 17 are rejected under 35 USC 101 because the claimed invention is directed to non-statutory subject matter.

The claims recite "A host cell comprising" which reads on a human being. Amending the claim to recite "An isolated cell" will obviate the rejection.

8. Claim 100 is rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter.

Claim 100 is drawn to a harvestable part of a plant of claim 98 or 99. The Office interprets "harvestable part" to read on seeds. Claims 98 and 99 are drawn to a transgenic plant comprising a transgenic rootstock. The office interprets claim 98 to be a plant with a transgenic rootstock which means that the scion, or upper part of the plant, is not necessarily transgenic. Therefore, seeds that are produced by the scion, will not necessarily be transgenic and will be indistinguishable from seeds that occur in nature. In the event that the scion is also transgenic, then due to Mendelian inheritance of genes, a single gene introduced into a parent plant would only be transferred at most to half the male gametes and half the female gametes. This translates into only three quarters of the progeny having at least a single copy of the transgene and one quarter of the progeny would not carry a copy of the transgene. Given that there is no indication that there would be any other distinguishable characteristics of the claimed progeny (seeds), it is

unclear whether the claimed seeds would be distinguishable from seeds that would occur in nature. See *Diamond v. Chakrabarty*, 447 U.S. 303 (1980), *Funk Bros. Seed Co. v. Kalo Inoculant Co.*, 333 U.S. 127, 76 USPQ 280 (1948), and *In re Bergy , Coats, and Malik* 195 USPQ 344, (CCPA) 1977.

Claim Rejections - 35 USC § 102

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Assuming Applicants are enabled for the full breadth of their claims, then this rejection is set forth.

9. Claims 2-4, 7-17, 25, 28-44, 49-50, 52-53, 79-81, 86-87, 90-92, 95-101, 103-121 and 138 are rejected under 35 U.S.C. 102(b) as being anticipated by Morris (February, 1999, WO 99/06571).

The claims are drawn to methods comprising an isolated nucleic acid molecule comprising a DNA sequence as set forth in SEQ ID NO:26, or the complement thereof, an isolated nucleic acid molecule comprising the RNA sequence corresponding to SEQ ID NO:26 or the complement thereof, a functional fragment of said nucleic acid molecule having the biological activity of a cytokinin oxidase; an isolated nucleic acid molecule encoding a plant protein having cytokinin oxidase activity selected from the group consisting of: an isolated nucleic acid molecule comprising a DNA sequence as set forth in SEQ ID NO:26, or the complement thereof, an isolated nucleic acid molecule comprising the RNA sequence corresponding to SEQ ID NO:26 or the complement thereof, a nucleic acid encoding a protein with an amino acid sequence comprising the polypeptide as given in SEQ ID NO:32 and which

is at least 70% similar to the amino acid sequence as given in SEQ ID NO:4, an isolated nucleic acid molecule encoding an immunologically active fragment or a functional fragment of a cytokinin oxidase encoded by a nucleic acid molecule as set forth in SEQ ID NO:26, or a immunologically active fragment or functional fragment of any of the above nucleic acid molecules, a nucleic acid molecule encoding a protein as defined in SEQ ID NO:4; or vector, host cell, composition or plant comprising said nucleic acid molecule; and methods of stimulating root growth, enhancing lateral or adventitious root formation, stimulating root growth, increasing root size, the production of a transgenic plant and delaying the onset to flowering, effecting the expression of a polypeptide, increasing the size of the root meristem, altering leaf senescence, increasing leaf thickness, decreasing vessel size, improving standability, increasing branching, improving lodging resistance, increasing seed size or weight, increasing embryo size or weight, increasing cotyledon size, increasing yield, increasing growth of seedling or an increase in early vigor, or increasing stress tolerance comprising expression of said nucleic acid molecule.

The Office interprets an isolated nucleic acid molecule comprising 'a' DNA sequence as set forth in SEQ ID NO:26 to read on a large number of sequences because the Office interprets "an isolated nucleic acid molecule comprising 'a' DNA sequence set forth in SEQ ID NO:26" to encompass nucleic acids comprising any portion of SEQ ID NO:26, including any dinucleotide of SEQ ID NO:26.

The Office interprets "an isolated nucleic acid molecule comprising the RNA sequence '*corresponding*' to SEQ ID NO:26, or complement thereof" to encompass RNA molecules

encoded by homologues of SEQ ID NO:26. Therefore claims 2b and 3b are drawn to a large number of sequences.

The Office interprets a nucleic acid molecule encoding '*a*' protein as defined in SEQ ID NO:4, or complement thereof, to read on a large number of sequences because the Office interprets "a nucleic acid molecule encoding '*a*' protein as defined in SEQ ID NO:4" to encompass nucleic acid encoding any portion of SEQ ID NO:4, including any two amino acids of SEQ ID NO:4.

Because Applicants have not defined "similar" and for purposes of this rejection, the Office interprets "similar" to encompass a multitude of sequences encoding any protein having cytokinin oxidase activity.

The Office interprets "functional fragment" and "immunologically active fragment" to mean a fragment comprising an epitope to which an antibody can bind (See Applicants' specification page 39, lines 27-29 and page 40, lines 16-17).

Applicants define "functional fragment" to "include those comprising an epitope which is specific for the proteins according to the invention" (page 40, lines 17-19).

Morris teaches a cytokinin oxidase cDNA from maize (ckx1) comprising SEQ ID NO:3 (page 23, lines 30-32), in a vector comprising a promoter that facilitates transcription and transformed into *Pichia pastoris* (page 26, Example 2) and transformed into tobacco using a constitutive and root specific promoter (page 29-32, Example 4). Given the Office's interpretation of "an isolated nucleic acid molecule comprising 'a' DNA sequence as set forth in SEQ ID NO:26", "an isolated nucleic acid molecule comprisnig the RNA sequence 'corresponding' to SEQ ID NO:26", "a nucleic acid molecule encoding 'a' protein as defined in

SEQ ID NO:4", and the Office's interpretation of "similar" as discussed above, then the sequence of Morris reads on the recited claims. In addition, based on Applicants' definition of "functional fragment" and "immunologically active fragment" as discussed above, the sequence of Morris reads on the recited claims. It would be an inherent property of a plant transformed with a plant cytokinin oxidase of Morris, that cytokinin activity or levels are reduced, root growth is stimulated and lateral and adventitious roots are formed, root size is increased, a transgenic plant is produced, flowering is delayed, the expression of a polypeptide is effected, the root meristem is increased, leaf senescence is altered, leaf thickness is increased, vessel size is decreased, standability and branching is improved, lodging resistance is improved, seed size or weight is increased, embryo size or weight is increased, cotyledon size and yield is increased, seedling growth or vigor is increased, and stress tolerance is increased, and as such, Morris anticipates the claimed invention.

10. Claims 3-4, 7, 10-11 and 14-15 are rejected under 35 U.S.C. 102(b) as being anticipated by Lin et al (January 1999, NCBI Accession Number AC005917).

The claims are drawn to an isolated nucleic acid molecule encoding a plant protein having cytokinin oxidase activity selected from the group consisting of: an isolated nucleic acid molecule comprising a DNA sequence as set forth in SEQ ID NO:26, or the complement thereof, an isolated nucleic acid molecule comprising the RNA sequence corresponding to SEQ ID NO:26 or the complement thereof, an isolated nucleic acid molecule specifically hybridizing to SEQ ID NO:26 or to the complement thereof under medium stringency conditions as specified in claim 2c, a nucleic acid encoding a protein with an amino acid sequence comprising the

polypeptide as given in SEQ ID NO:32 and which is at least 70% similar to the amino acid sequence as given in SEQ ID NO:4, an isolated nucleic acid molecule encoding an immunologically active fragment or a functional fragment of a cytokinin oxidase encoded by a nucleic acid molecule as set forth in SEQ ID NO:26, or a immunologically active fragment or functional fragment of any of the above nucleic acid molecules; a vector comprising said nucleic acid molecule and host cell transformed with said vector.

The Office interprets an isolated nucleic acid molecule comprising 'a' DNA sequence as set forth in SEQ ID NO:26 to read on a large number of sequences because the Office interprets "an isolated nucleic acid molecule comprising 'a' DNA sequence set forth in SEQ ID NO:26" to encompass nucleic acids comprising any portion of SEQ ID NO:26, including any dinucleotide of SEQ ID NO:26.

The Office interprets "an isolated nucleic acid molecule comprising the RNA sequence '*corresponding*' to SEQ ID NO:26, or complement thereof" to encompass RNA molecules encoded by homologues of SEQ ID NO:26. Therefore claims 2b and 3b are drawn to a large number of sequences.

The Office interprets a nucleic acid molecule encoding '*a*' protein as defined in SEQ ID NO:4, or complement thereof, to read on a large number of sequences because the Office interprets "a nucleic acid molecule encoding '*a*' protein as defined in SEQ ID NO:4" to encompass nucleic acid encoding any portion of SEQ ID NO:4, including any two amino acids of SEQ ID NO:4.

Because Applicants have not defined "similar" and for purposes of this rejection, the Office interprets "similar" to encompass a multitude of sequences encoding any protein having cytokinin oxidase activity.

The Office interprets "functional fragment" and "immunologically active fragment" to mean a fragment comprising an epitope to which an antibody can bind (See Applicants' specification page 39, lines 27-29 and page 40, lines 16-17).

Applicants define "functional fragment" to "include those comprising an epitope which is specific for the proteins according to the invention" (page 40, lines 17-19).

Lin et al disclose a nucleic acid molecule encoding a protein as defined in SEQ ID NO:4, wherein the nucleic acid molecule is taught in Genbank accession number: AC005917 (see enclosed sequence search results). The Office contends that the sequence of Lin et al encodes SEQ ID NO:4 and that the mis-coding of Alanine 230 and Lysine 405 is due to sequencing errors which are known to occur in DNA sequencing reactions. The nucleic acid molecule encodes a protein having cytokinin oxidase activity and encodes the protein as defined in SEQ ID NO:4. The nucleic acid sequence of Lin et al is contained in a vector and for purposes of molecular biology would be transformed into a host cell, and as such, Lin et al anticipate the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

11. Claims 3-4, 7-17, 25, and 30-42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lin et al (January 1999, NCBI Accession Number AC005917).

The claim is drawn to an isolated nucleic acid molecule encoding a plant protein having cytokinin oxidase activity comprising a nucleic acid molecule encoding a protein as defined in SEQ ID NO:4, or the complement thereof, provided that said nucleic acid molecule is not the nucleic acid molecule as deposited under Genbank accession number:AC005917; a vector comprising said nucleic acid molecule and host cell transformed with said vector, or wherein said vector is an expression vector wherein the nucleic acid molecule is operably linked to one or more control sequences allowing expression in a prokaryotic or eukaryotic host cell; or a method for producing a transgenic plant comprising said nucleic acid molecule in an expressible format or vector, or transgenic plant or harvestable part comprising said nucleic acid molecule.

The teachings of Lin et al have been disclosed above.

Lin et al do not teach an isolated nucleic acid molecule encoding a plant protein having cytokinin oxidase activity comprising a nucleic acid molecule encoding a protein as defined in SEQ ID NO:4, wherein the nucleic acid molecule is not the nucleic acid molecule deposited under Genbank accession number:AC005917 or vector comprising said nucleic acid molecule

operably linked to a regulatory element allowing expression in a plant, and a transgenic plant or harvestable part comprising said nucleic acid sequence and regulatory element.

Given the teachings of Lin et al of a nucleic acid molecule encoding a protein having cytokinin oxidase activity, it would have been obvious to one of ordinary skill in the art to isolate or make another nucleic acid molecule encoding a protein as defined in SEQ ID NO:4. It would have been further obvious to construct a vector comprising said nucleic acid molecule operably linked to a regulatory element operable in plants, and to transform a plant with said vector.

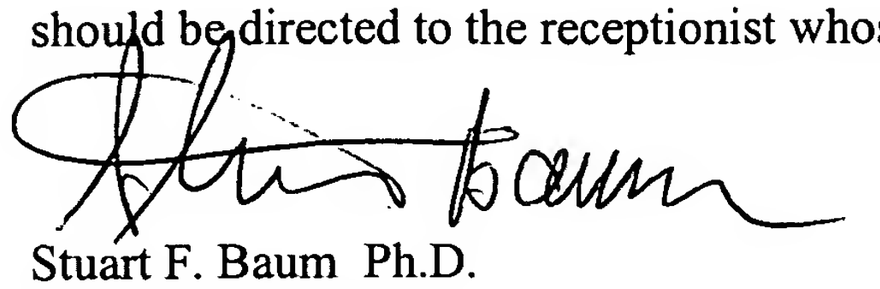
Thus the claimed invention would have been *prima facie* obvious as a whole to one of ordinary skill in the art at the time it was made, especially in the absence of evidence to the contrary.

12. No claims are allowed.

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stuart F. Baum whose telephone number is 571-272-0792. The examiner can normally be reached on M-F 8:30-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anne Marie Grunberg can be reached at 571-272-0975. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.



Stuart F. Baum Ph.D.
Patent Examiner
Art Unit 1638
January 4, 2006

Continuation of Disposition of Claims: Claims withdrawn from consideration are 5,6,18-24,26,27,45,48,51,54-78,82-85,88,89,93,94,102 and 122-137.

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            Nierman,W.C., Fraser,C.M. and Venter,J.C.
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 92822)
  AUTHORS   Lin,X.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-MAR-2000) The Institute for Genomic Research, 9712
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  TITLE     Direct Submission
  JOURNAL   Submitted (27-FEB-2002) The Institute for Genomic Research, 9712
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Alignment Scores:

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Query Match:	85.77%	Indels:	499
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US-10-014-101-4 (1-501) x AC005917 (1-92822)

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            rosids; eurosids II; Brassicales; Brassicaceae; Arabidopsis.
REFERENCE   1
  AUTHORS   Schmuelling,T. and Werner,T.
  TITLE     Method for modifying plant morphology, biochemistry and physiology
  JOURNAL   Patent: WO 0196580-A 28 20-DEC-2001;
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